

Research Note

Changes in the Antigenic and Immunoglobulin E–Binding Properties of Hen’s Egg Albumin with the Combination of Heat and Gamma Irradiation Treatment

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ABSTRACT

This study was carried out to evaluate the changes in the allergenic and antigenic properties of hen’s egg albumin (ovalbumin [OVA]) with the combination of heat and gamma irradiation treatment. OVA solution samples were treated by (i) heating (sample 1), (ii) irradiation after heating (sample 2), and (iii) heating after irradiation (sample 3). Samples were isothermally heated and irradiated at the absorption dose of 10 kGy. Competitive indirect enzyme-linked immunosorbent assays (ELISAs) were performed with blood serum to test the ability of treated OVA to bind to immunoglobulin E (IgE) and mouse murine monoclonal antibody (IgG). OVA’s ability to bind to mouse IgG changed upon heating at 75°C, and its ability to bind to egg-allergic IgE changed upon heating at 80°C. The ELISAs showed that egg-allergic IgE did not recognize OVA very well when heated at $\geq 80^\circ\text{C}$, while mouse IgG retained better activity under these conditions. Egg-allergic IgE binding was low both for OVA samples treated by heating and for samples treated by irradiation followed by heating. These results show that allergies induced by OVA could be effectively reduced by the combination of heat and gamma irradiation treatment.

A clinically abnormal response to an ingested food or food additive, generally known as an adverse reaction to food, may be mediated by immunologic, pharmacologic, toxic, infectious, idiosyncratic, metabolic, and neuropsychologic mechanisms (14, 20). The egg is one of the most allergenic foods, and minute amounts of egg can result in symptoms within minutes, including life-threatening anaphylaxis (9). The egg white appears to be more allergenic than the egg yolk. Among the egg white proteins, ovalbumin (OVA) and ovomucoid have been recognized as major allergens (15). Even though enzymatic hydrolysis with several proteolytic enzymes has been introduced and its limited use has been successful for purposes such as dried-milk processing (1, 17, 18), this method cannot be applied to the reduction or inhibition of egg allergies.

Meanwhile, food irradiation has been used for ensuring hygienic quality and for prolonging the shelf stability of food items (19). The structural modification of food allergens by gamma irradiation has recently been observed in several studies, and many results have indicated that ionizing radiation could reduce allergenicity or antigenicity through the destruction of binding epitopes of food proteins (2, 3, 8, 10). Moreover, the theory was practically applied, and it was found that the ability of OVA to bind to im-

munoglobulin E (IgE) in egg-allergic individuals was reduced by irradiation (12). Most foods are prepared by cooking. It has been reported that the boiling of egg whites decreased allergenicity and abrogated the intestinal uptake of immunoreactive OVA in mice (6, 13). Therefore, we hypothesize that the combination of irradiation and heat treatment may enhance the reduction of allergenicity profoundly.

This study was conducted to evaluate the effectiveness of gamma radiation in reducing egg allergenicity by observing the changes in the antigenic and IgE-binding properties of OVA with the combination of heat and irradiation treatment.

MATERIALS AND METHODS

Protein and antibodies. Isolated hen’s egg albumin (OVA) was purchased from Sigma Chemical Co. (St Louis, Mo.) for use with the standard antigen and the standard allergen of egg white protein. Murine monoclonal antibody (M-IgG) against chicken egg albumin was also purchased from Sigma. Human sera were obtained from 22 patients <3 years of age (15 boys and 7 girls) diagnosed with IgE-mediated egg allergy as indicated by open egg challenges and/or a convincing history of acute urticaria or diarrhea after egg ingestion. Sixteen patients had atopic dermatitis and/or acute urticaria, five patients had skin and gastrointestinal symptoms (diarrhea and vomiting), and two patients had only chronic diarrhea. All patients showed elevated egg-specific IgE

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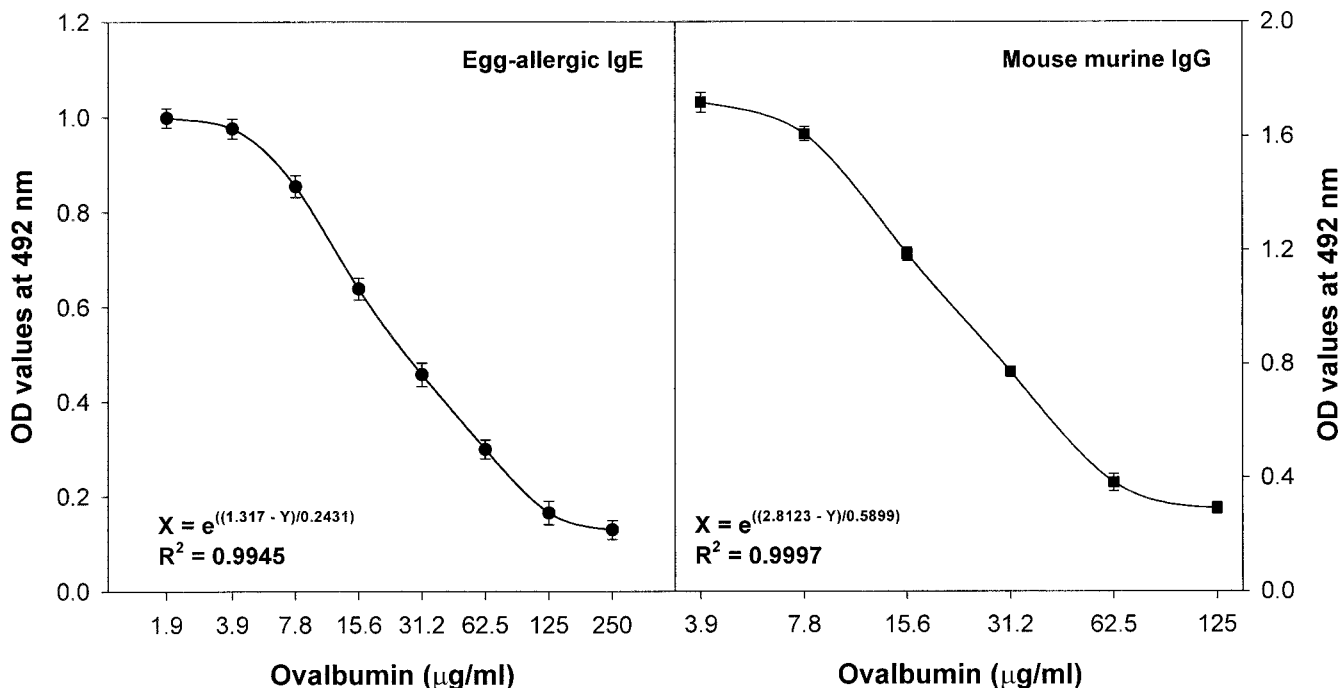


FIGURE 1. Standard curves for the quantification of OVA. The curves were determined by Ci-ELISA individually formatted with egg-specific IgE (left) and M-IgG (right) against OVA. The standard solution was prepared by dilution of untreated OVA solution (2.0 mg/ml).

(0.7 to 100 kU/liter), as measured by AlaSTAT RIA (DPC Co., Los Angeles, Calif.) or Pharmacia CAP System FEIA (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden), and the sera were pooled to determine the IgE-binding ability of OVA undergoing different treatments. Ten age-matched subjects provided serum for controls. None of the control subjects had food allergies or any other atopy-related skin, respiratory, or gastrointestinal symptoms. As secondary antibodies (Ab) with chromogenic enzymes, horseradish peroxidase conjugated rabbit anti-human IgE IgG (Sigma Chemical Co.) and horseradish peroxidase conjugated rabbit anti-mouse IgG IgG (Sigma Chemical Co.) were used for egg-allergic IgE and M-IgG, respectively.

Sample preparation. OVA was dissolved in 0.01 M sodium phosphate-buffered saline (PBS) with 0.15 M NaCl (pH 7.4) and adjusted to a final concentration of 2.0 mg/ml. The solution was divided into three portions, sample 1 (treated with heat), sample 2 (treated with irradiation after heating), and sample 3 (treated with heat after irradiation).

Heat treatment. Intact OVA solution (5 ml) and irradiated OVA solution (5 ml) were individually put into glass tubes (diameter, 1.0 cm; glass thickness, 1 mm) with caps, and 11 tubes were prepared. Each of nine tubes was placed in a water bath (W 400, Büchi, Switzerland) equipped with a thermocouple (CTF 9008, Ellab, Denmark) and isothermally heated for 15 min at 37, 60, 70, 75, 80, 85, or 90°C. One of the remaining tubes was heated and the other was autoclaved under sterile conditions. After heating, the tubes were cooled in iced water for 15 min and then stored at 4°C.

Gamma irradiation. Intact OVA solution and heated tubes with OVA solution were irradiated in a cobalt-60 irradiator (IR-79, Nordion International Ltd., Ontario, Canada) equipped with 100-kCi activity at $10 \pm 0.5^\circ\text{C}$ and operated at a dose rate of 10 kGy/h. The applied dose was 10 kGy. The absorbed dose was monitored with both free-radical and ceric/cerous dosimeters. Af-

ter irradiation, the heated tubes were stored at 4°C, and irradiated OVA solution (5 ml) was put into a glass tube and heated and cooled by the method described above.

Competitive indirect ELISA. Competitive indirect enzyme-linked immunosorbent assays (Ci-ELISAs) were individually formatted with pooled sera obtained from 22 children with egg-allergic patient IgE antibodies (P-IgE, ≥ 6 kU/ml or more by CAP-RAST [Radio Allergo Sorbent Test]) and M-IgG by previously reported methods (11) with slight modifications. Briefly, polystyrene flat-bottom microtiter plates (Maxisorp, Nunc, Kamstrup, Denmark) were coated with 100 µl of untreated OVA solution (10.0 µg/ml for P-IgE and 1.0 µg/ml for M-IgG) in a 0.2 M bicarbonate buffer (pH 9.6) overnight at 4°C. All subsequent steps were carried out at 37°C. Plates were washed three times with PBS containing 0.05% (vol/vol) Tween 20 (PBST). To reduce nonspecific binding, the plates were blocked by incubation for 1.5 h with 120 µl of PBS containing 1% (wt/vol) gelatin (75 to 100 bloom, type A from porcine skin; Sigma). After washing, 50 µl of standard solution or sample solution was added to coated and blocked wells, and then 50 µl of each Ab solution was added. The plates were incubated for 2 h and then washed three times with PBST. After the addition of 100 µl of secondary Ab solution to the wells, the plates were incubated for 1.5 h. The plates were then washed, and 100 µl of 0.04% *o*-phenylenediamine (Sigma) in 0.1 M phosphate-citrate buffer (pH 5.0) with 0.04% (vol/vol) hydrogen peroxide (35% H_2O_2) was added for color reaction for 20 min, and then the reaction was stopped with 2.0 M H_2SO_4 (50 µl per well). The absorbance was measured at 492 nm with an ELISA reader (CERES UV-900C, BIO-TEK Instruments Inc., New York, N.Y.).

For standards, untreated OVA solution was serially prepared in concentrations of 0.12 to 1,000 µg/ml, and sample solutions were individually diluted to 20.0 µg/ml in PBS. P-IgE was diluted 25 times in PBS, and M-IgG was diluted 5,000 times in PBS.

The concentration of intact OVA in diluted samples was de-

TABLE 1. Egg-specific IgE- and mouse murine IgG-binding capacities^a (detected concentration, µg/ml) of each OVA sample treated with gamma irradiation of 10 kGy and/or heat

Type of Ab	Treatment ^b	Concn at heating temp (°C) ^c							
		37	60	70	75	80	85	90	Autoclaved
Egg-specific IgE	Sample 1	10.3 ± 0.8	9.9 ± 0.6	10.5 ± 0.7	9.5 ± 0.7	6.1 ± 0.7	1.9 ± 0.6	1.2 ± 0.4	1.3 ± 0.5
	Sample 2	1.5 ± 0.6	1.2 ± 0.5	1.7 ± 0.7	1.4 ± 0.7	1.4 ± 0.6	0.5 ± 0.2	0.4 ± 0.3	0.5 ± 0.3
	Sample 3	1.8 ± 0.5	1.4 ± 0.6	1.3 ± 0.6	0.9 ± 0.5	0.9 ± 0.3	0.7 ± 0.1	0.9 ± 0.2	1.0 ± 0.2
Mouse murine IgG	Irradiation	1.5 ± 0.2							
	Control	9.7 ± 0.4							
	Sample 1	11.2 ± 0.8	12.1 ± 1.2	17.1 ± 14	121.4 ± 9.6	258.3 ± 6.7	346.9 ± 14.6	289.9 ± 12.7	204.4 ± 4.5
	Sample 2	49.5 ± 1.6	52.2 ± 4.1	57.4 ± 3.9	52.3 ± 5.2	47.6 ± 2.3	43.4 ± 7.5	40.3 ± 3.7	36.1 ± 2.5
	Sample 3	52.2 ± 3.1	69.5 ± 4.5	76.4 ± 3.6	82.8 ± 1.9	92.6 ± 4.6	107.34 ± 4.8	111.9 ± 8.2	100.5 ± 7.6
	Irradiation	181.1 ± 7.3							
	Control	10.1 ± 0.6							

^a Mean ± standard deviation (*n* = 5).^b Sample 1, heating; sample 2, irradiation after heating; Sample 3, heating after irradiation; Irradiation, irradiation at 10°C with absorbed dose of 10 kGy; Control, untreated OVA maintained with mild shaking at room temperature (21°C) for 15 min.^c Samples were isothermally heated to temperatures of up to 95°C in a waterbath for 15 min, and the temperatures of samples heated in boiling water for 15 min and autoclaved in the sterilized condition were 100 and 121°C, respectively.

terminated by the standard curve of each Ab. The standard curve was determined by the reaction of Ab to intact OVA in Ci-ELISA.

Statistical analysis. All samples were tested in triplicate, and the experiments were repeated five times. Means and standard deviations calculated from the equations for egg-specific IgE and M-IgG were used to evaluate the detected concentration of OVA. The data were analyzed by general linear procedures, least-squares means, and Duncan's multiple-range test. SAS software (16) was used for the analysis.

RESULTS AND DISCUSSION

Standard curves for quantifying antibody-binding capacity of OVA samples. Standard curves were individually obtained with Ci-ELISA formatted with P-IgE and M-IgG to quantify OVA in sample solution (Fig. 1). In Ci-ELISA with P-IgE, OVA could be determined at 3.9 to 125 µg/ml, and the concentration of OVA could be calculated by the equation $x = e^{(1.317-y)/0.2431}$, obtained from the curve, where *x* is the concentration of OVA in sample solutions and *y* is the average optical density value of microwells of sample solution. In Ci-ELISA with M-IgG, OVA could also be quantified at 7.8 to 62.5 µg/ml in the standard curve, and the equation of the curve was $x = e^{(2.8123-y)/0.5899}$.

Changes in IgE- and IgG-binding capacities of OVA samples treated with irradiation and/or heat. Table 1 shows changes in the capacity of OVA to bind to Ab in sample solutions. It has been reported that OVA was thermally denatured from about 70°C and that the denaturation was highest at 80 to 85°C for neutral pH (4, 7). The capacities of OVA to bind to M-IgG and P-IgE started to change at 75 and 80°C, respectively. P-IgE did not recognize OVA heated at >80°C well. This finding is supported by a previous report that indicated that the antigenicities of OVA and several food allergens were reduced by heat treatment (6, 13). M-IgG recognized OVA heated to >75°C better, and the immune reaction was strongest at 85°C with a reactivity of about 34.7 times. A protein can be altered by heating, and its antigenicity can also be changed (5). The conformational releases can be induced by mild heating, and it is possible that this explains why more M-IgG is bound (11).

Allergenicities were very low for both sample 2 and sample 3. The allergenicity of OVA did not increase with the combination of irradiation and heat treatment; it even decreased slightly. The antigenicity of OVA irradiated at 10 kGy increased, as noted in a previous report (12). Isothermal heat treatment induced an increase in antigenicity for sample 3, but such an increase was not observed for sample 2.

No studies on the allergenic and antigenic properties of other food allergens or on OVA treated with the combination of irradiation and heat have been reported to date. Further study is needed to elucidate these data. Our data indicate that irradiation might reduce the allergenicity of foods.

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